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# Electron Capture Gas Chromatographic Analysis of the Amine Metabolites of Pesticides: Derivatization of Anilines

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16. ABSTRACT

A number of amines have been shown to result from metabolism of various pesticides. From an epidemiological standpoint, it may be possible to monitor human exposure to these pesticides through the excretion of their corresponding amines in urine. An investigation has been initiated to develop and apply methods of analysis of amines in human urine. The results of a survey of derivatization techniques involving several substituted amines are presented. These include conditions for derivatization, utilizing a number of halo- and nitro-substituted reagents; electron capture and gas chromatographic properties of the derivatives; and stability of the derivatives to extraction and column chromatography for purposes of separation and cleanup. The recoveries of anilines from spiked water and urine samples at the 1.0 ppm and 0.1 ppm levels were between 85 and 90%. The advantages and disadvantages of the various derivatives and techniques are discussed and a rationale is presented for the preliminary selection of a particular derivative for application of the analysis of aniline metabolites in urine.

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# Electron Capture Gas Chromatographic Analysis of the Amine Metabolites of Pesticides: Derivatization of Anilines*

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## Abstract

**A number of amines have been shown to result from metabolism of various pesticides. From an epidemiological standpoint, it may be possible to monitor human exposure to these pesticides through the excretion of their corresponding amines in urine. An investigation has been initiated to develop and apply methods of analysis of amines in human urine. The results of a survey of derivatization techniques involving several substituted anilines are presented. These include conditions for derivatization, utilizing a number of halo- and nitro- substituted reagents; electron capture and gas chromatographic properties of the derivatives; and stability of the derivatives to extraction and column chromatography for purposes of separation and cleanup. The recoveries of anilines from spiked water and urine samples at the 1.0 ppm and 0.1 ppm levels were between 85 and 90%. The advantages and disadvantages of the various derivatives and techniques are discussed and a rationale is presented for the preliminary selection of a particular derivative for application of the analysis of aniline metabolites in urine.**

## Introduction

As the persistent pesticides are being phased out and readily degradable compounds take their place, there is a rising demand for methods for the analysis of degradation products of these compounds in addition to the intact pesticides themselves. An appreciable portion of today's commonly used pesticides now contains amine functions, either aromatic or aliphatic, which may be released into the environment by metabolic or degradative processes. Therefore, a real need exists for methods applicable to the analysis of mixtures of amines in environmental substrates.

The anilines were the first group of amines to be considered in the present study. They are possible metabolic and degradation products of a number of amide, carbamate and urea pesticides, shown in Table I. A method for the analysis of any or all of these anilines was needed. It was felt that the most satisfactory approach was via gas chromatography of a volatile derivative. Because of the low levels involved, the electron capture detector was the detector of choice, and the aniline derivative must therefore have good electron capturing properties. This can be accomplished by choosing a derivatizing reagent that is halo and or nitro substituted. Fortunately,

Table I. Anilines arising from degradation of various pesticides.

| Class | Pesticide | Proposed Degradative Product |
|---|---|---|
| Amide | Propanil | 3,4-dichloroaniline |
| Carbamate | Propham (IPC) | Aniline |
| | Carbetamide | Aniline |
| | Chloropropham (CIPC) | m-chloroaniline |
| | Barban | m-chloroaniline |
| Urea | Fenuron | aniline |
| | Siduron | aniline |
| | Monuron | p-chloroaniline |
| | Monolinuron | p-chloroaniline |
| | Linuron | p-chloroaniline |
| | Metabromuron | p-bromoaniline |
| | Diuron | 3,4-dichloroaniline |
| | Neburon | 3,4-dichloroaniline |
| | Linuron | 3,4-dichloroaniline |
| | Chlortoluron | 3-chloro-4-methyl-aniline |

the literature contains a number of references to reagents meeting this requirement. These derivatization reactions generally produce one of two classes of products: amides formed from acid chlorides, anhydrides or imidazoles; and substituted amines, resulting from reaction with alkyl or aryl halides.

Amides result from the reaction of trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) with amines, as described by Clark et al. (1), Cummins and Fourier (2), and Vessman et al. (3). Heptafluorobutyrylimidazole (HFBI) reacts with amines to produce the same product as heptafluorobutyric anhydride [Sisenwine et al. (4), and Horning et al. (5)].

Pentafluorobenzoyl chloride and pentafluorophenylacetyl chloride were used by Moffat et al. (6) to produce amide derivatives of primary aliphatic amines. Tilden and van Middelem (7) similarly used *p* bromobenzoyl chloride to produce amides from amines.

Substituted amines were formed from pentafluorobenzyl bromide and primary aliphatic amines by Moffat et al. (6). Kawahara (8,9) had previously used this reagent to form esters and ethers from organic acids and phenols respectively. A number of nitro- and trifluoromethyl-substituted fluorobenzenes were reacted with primary amines by Day et al. (10), Crosby and Bowers (11), Holden et al. (12), and Cohen and Wheals (13) to give secondary amine derivatives.

With this variety of reagents available, it was necessary to evaluate the utility of each technique to the analysis of amine mixtures. The factors to be kept in mind in the evaluation were:

1. Stability, purity, ease of handling and cost of reagents.
2. Reaction conditions, completeness of reaction, ease of separation of derivative from unreacted reagent and reaction products.

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JOURNAL OF CHROMATOGRAPHIC SCIENCE•VOL. 15
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3. Stability of derivative to extraction and cleanup by column adsorption chromatography.
4. Gas chromatographic characteristics, response, resolution of mixture of anilines, thermal stability of derivatives, time required for complete elution of peaks, interfering peaks resulting from reagents or substrate.

## Experimental

*Apparatus and Glassware*: All mixing was accomplished using a Vortex Genie Mixer (Scientific Industries, Inc.) or a Roto-rack Model 96 (Fisher Scientific Co.). Special glassware included 13 ml graduated, glass-stoppered centrifuge tubes and size 23 Chromaflex columns available from Kontes, and 16 × 125 mm culture tubes with Teflon-lined screw caps.

Gas chromatography was on a Micro-Tek MT-220 gas chromatograph equipped with a tritium foil electron capture detector. All columns were of borosilicate glass, 1.82 meters long, 6.4 mm o.d., 4 mm i.d. Off-column injection was used. The columns were packed, heat conditioned and evaluated as described in the *Manual of Analytical Methods for the Analysis of Pesticide Residues in Human Environmental Samples* [Thompson (14)]. The carrier gas was nitrogen. The detector was operated at 215° C, and the inlet and transfer lines were operated at 225° C.

Applied Science was the source of the following prepacked column packings: 5% OV-210 on 80–100 Gas-Chrom; 4% SE-30/6% QF-1 on 80–100 Gas-Chrom Q (discontinued); 4% SE-30/6% OV-210 on 80/100 Gas Chrom (replaces 4% SE-30/6% QF-1); and 3% OV-1 on 60/80 Gas-Chrom Q. These column packings were either stock packings or custom-made packings.

The following column packings were prepared in the laboratory: 5% SE-30 on 80–100 Gas-Chrom Q; 2% OV-17/8% OV-210 on 80–100 Gas Chrom Q; and 1% Silicone E-301/0.1% Epon 1001 on 80–100 Gas Chrom Q. The slurry technique was used and the solvent removed with a rotatory evaporator using a Morton flask.

Mass spectra of aniline derivatives were obtained on a Hewlett-Packard 5930A mass spectrometer equipped with a model 5700A gas chromatograph. The mass range from 50 to 500 m/e was scanned at a rate of 160 m/e/sec, and the data was recorded in a Hewlett-Packard model 5933A Data System. Spectra were obtained by temperature programming, from 100° C (2 min) to 230° C at 8° C/min. A 183 cm × 2 mm i.d. glass column packed with 1.5% OV-17/1.95% OV-210 on 80–100 mesh Gas Chrom Q was used. The GC inlet temperature was 200° C. The transfer line to the mass spectrometer was maintained at 210° C. The membrane separator was located in the column oven and hence the temperature was programmed when the column was programmed. The helium flow rate was 42 ml/min.

*Reagents*: Aniline, certified ACS, was obtained from Fisher Scientific Company. Aniline hydrochloride, *m*-chloroaniline (practical), *p*-chloroaniline, *p*-bromoaniline, *p*-bromoaniline hydrochloride, 3,4-dichloroaniline, and *p*-bromobenzoyl chloride (BBC), were obtained from Eastman Organic. Aldrich Chemical Co. was the source of pentafluorobenzoyl chloride, pentafluorobenzyl bromide (PFB, α-bromo-2,3,4,5,6-pentafluorotoluene), 1-fluoro-2,4-dinitrobenzene (FDNB, 2,4-dinitro-fluorobenzene), and 3-chloro-4-methylaniline. Pentafluoropropionic anhydride (PFPA), heptafluorobutyric anhydride (HFBA) and heptafluorobutyryl imidazole (HFBI) were obtained from Pierce Chemical Co. J. T. Baker Chemical Co. provided *p*-bromobenzoyl bromide (BBB), 4-chloro-α,α,α-trifluoro-3,5-dinitrotoluene (CDNT), and α,α,α,4-tetrafluoro-3-nitrotoluene (FMNT). These reagents were all used as received, without purification.

Anhydrous sodium sulfate was extracted in a Soxhlet apparatus for eight hours with benzene, dried and stored in a 130° oven. Woelm silica gel, Activity grade I, was obtained from Waters Associates, Inc. It was activated for at least 24 hours in a 130° oven before use. A portion for use in the analysis was partially deactivated by weighing into a vial with a Teflon-lined screw cap and adding a measured amount of water to give the percent water indicated in the method. This was placed on the Rotorack for one hour at 50 rpm. The prepared adsorbent could be stored in a tightly closed container for at least a week.

## Reactions of Standards

1. *Amides from anhydrides and imidazoles*: Weigh about 1 mg of the aniline or its hydrochloride salt into a 13 ml graduated, conical centrifuge tube. Add 0.1 ml of the anhydride (PFPA or HFBA) or imidazole (HFBI), stopper and allow the solution to stand with occasional mixing for 1 hour at room temperature. If necessary, heat gently to dissolve the aniline, then allow to stand at room temperature. Alternatively, dilute the weighed aniline to a known volume with benzene. Pipette 0.1 ml of the solution, equivalent to about 0.5 μg, into a 13 ml centrifuge tube. Add 0.1 ml of ethyl acetate and 0.1 ml of anhydride or imidazole, mix and allow to stand for one hour at room temperature.

At the end of one hour, add 2 ml of water to the anhydride reaction mixture or 2 ml of dil. HCl (1 ml conc. HCl diluted to 100 ml with water) to the imidazole reaction mixture. Add four ml of benzene, mix on Vortex mixer for one minute, centrifuge to separate layers and discard the lower aqueous layer. Wash the benzene extract twice with 2 ml portions of water, discarding the aqueous washes. Dilute the benzene solution of aniline derivatives to 10 ml, using the graduations on the centrifuge tube. Add a little anhydrous $Na_2SO_4$ to remove traces of water, and make further dilutions if necessary to give a working standard at a concentration of about 50 ng/ml.

*Discussion*: The reaction proceeded to completeness within one hour at room temperature. Heating was not necessary and, if anything, somewhat decreased the peak heights. The reaction gave the same results whether run in solution or heat and whether the free aniline or its HCl salt was used. Completeness of reaction and extraction were demonstrated by isolating and recrystallizing a quantity of some of the derivatives, making up standard solutions from these pure derivatives, and comparing them gas chromatographically to the extract from the derivatization reaction.

The derivative was subjected to cleanup by adsorption chromatography using one gram of silica gel (deactivated with 3% water) in a size 23 Chromaflex column. Recovery was complete, all amides eluting from the column with 15 ml of 60% benzene in hexane (after a preelution with 10 ml of 20% benzene in hexane to remove the less polar interfering compounds).

A number of gas chromatographic columns were tried, mainly using silicone stationary phases, including 5% OV-210 at 160° C, 5% SE-30 at 150° C, 4% SE-30/6% QF-1 at 160° and 175°, and 4% SE-30/6% OV-210 at 150° C, all with a carrier flow of 50–60 ml/min. Also tried was a column of 2% OV-17/8% OV-210 at 150° C and either 40 ml/min or 70 ml/min carrier flow. With none of these columns was it possible to obtain better than minimal resolution of *m*-chloro- and *p*-chloroaniline derivatives. Figures 1 and 2 show chromatograms of amide derivatives of anilines.

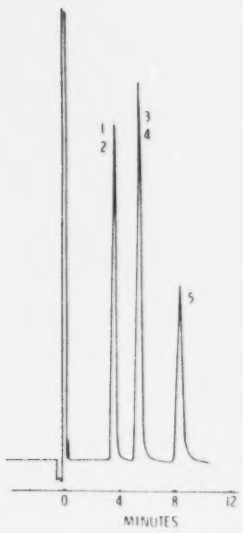



Figure 1. Chromatogram of heptafluorobutyramide derivatives of substituted anilines, using a 6 ft 5% OV-210 column at 160°C, 60 ml/min carrier flow and an attenuation of 8 x 10; 50 pg each of 1) *m*-chloroaniline, 2) *p*-chloroaniline, 3) 3-chloro-4-methylaniline, 4) *p*-bromoaniline, and 5) 3,4-dichloroaniline.

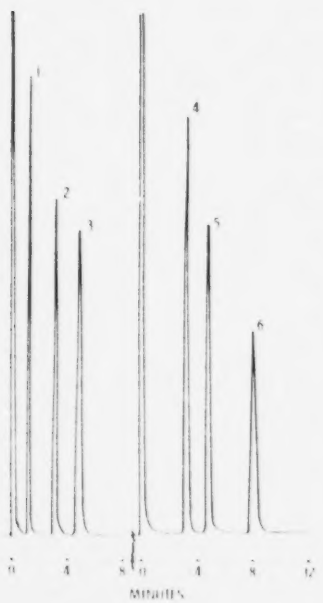



Figure 2. Chromatogram of pentafluoropropylamide derivatives of substituted anilines using a 6 ft 5% OV 210 column at 155°C, 50 ml/min carrier flow and an attenuation of 8 x 10; 50 pg each of 1) aniline, 2) *m* chloroaniline, 3) 3 chloro 4 methylaniline, 4) *p*-chloroaniline, 5) *p*-bromoaniline, and 6) 3,4 dichloroaniline.

Although HFBA and HFBI gave the same products upon reaction with anilines, there were a number of factors favoring the anhydride. The reaction using HFBI seemed to go to only 80-85% of completion compared to HFBA reaction. HFBA appeared to be a much purer reagent, both upon visual inspection and on evidence of reagent blanks. HFBA gave a very clean reagent blank with a sharp, narrow "solvent" front, whereas, HFBI produced a broad, tailing "solvent" front and numerous later eluting peaks. A wash with dilute HCl was necessary to remove a crystalline product of the degradation of excess HFBI. This narrowed the "solvent" peak somewhat, but the chromatogram was still inferior to that obtained with HFBA.

The response and retention time of corresponding PFP and HFB amides were remarkably similar, as seen in Figures 1 and 2. The PFPA reagent produced a cleaner reagent blank and for this reason, had an advantage over HFBA.

II. *Amides from p-bromobenzoyl bromide (BBB)*: Prepare aniline standards in acetone solution at a concentration of about 0.2 mg/ml. Pipette 0.1 ml (equivalent to about 20 μg) into a 16 × 25 mm culture tube with a Teflon-lined screw cap. Add 5 ml of water, 5.0 ml of *p*-bromobenzoyl bromide reagent (0.1 ml of BBB in 100 ml of benzene) and 1 ml of NaOH (15% in water). Place on the Roto-Rack for 10 minutes at 50 rpm. At the end of this time, allow the layers to separate, centrifuging if necessary. Dilute an aliquot of the benzene layer to give a working standard at a concentration of about 0.2 μg/ml in benzene.

*Discussion:* Although Tilden and van Middelem (7) report derivatization of aliphatic amines using *p*-bromobenzoyl chloride (BBC), we found no derivatization of anilines with the BBC reagent available to us. Using *p*-bromobenzoyl bromide, the reaction proceeded with no difficulty.

The derivatives were stable to silica gel column chromatography. Complete recovery was obtained through a column containing 1 g silica gel, partially deactivated with 5% water. A preelution with 10 ml of 40% benzene in hexane was discarded and the amides eluted from the column with 15 ml of benzene.

As with the aliphatic amides just discussed, it was difficult to find a gas chromatographic column which would resolve *p*-chloro- and *m*-chloroaniline derivatives. The compounds eluted very slowly from columns packed with 5% SE-30, 5% OV-210 or 3% OV-1, each at 200°C and a carrier flow of 75 ml/min. Because of the long retention times, temperatures and flows could not be significantly reduced. Minimal resolution was obtained using a 1% Silicon E-301/0.1% Epon 1001 stationary phase at 180°C and 80 ml/min carrier flow, as shown in Figure 3.

III. *Amides from pentafluorobenzoyl chloride:* The pentafluorobenzamide derivatives of anilines were prepared by three methods:

(a) Evaporate 0.1 ml of an acetone solution of the aniline standard (equivalent to 10-20 μg) to dryness. Dissolve the residue in 0.2 ml of acetonitrile, add 0.1 ml of pentafluorobenzoyl chloride, and heat in a sealed tube for 1 hour at 60°C. Cool, dilute to 10 ml with hexane. Make a further dilution of 1 ml to 10 ml.

(b) Follow the same procedure as described for the preparation of *p*-bromobenzamides, using a reagent made by dissolving 0.1 ml of pentafluorobenzoyl chloride in 50 ml benzene.

(c) Use Procedure (b) but omit the 1 ml of NaOH solution.

*Discussion:* The derivatization of anilines with pentafluorobenzoyl chloride was more complex than derivatization with any other reagent studied. When a mixture of six anilines was

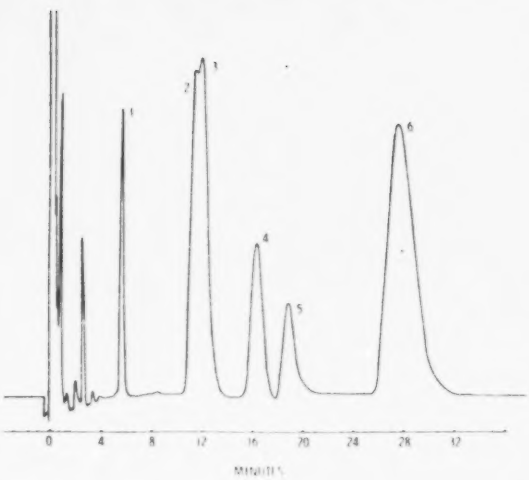



Figure 3. Chromatogram of *p*-bromobenzamide derivatives of substituted anilines using a 6 ft 1% E-301/ 0.1% Epon 1001 column at 180°C, 80 ml/min carrier flow and an attenuation of 16 x 10; 0.5 ng of 1) aniline, 1 ng each of 2) *m*-chloroaniline, 3) *p*-chloroaniline, 4) 3-chloro-4-methylaniline and 5) *p*-bromoaniline and 2 ng of 6) 3,4-dichloroaniline.

derivatized, gas chromatography showed ten peaks which were not present in the reagent blank.

Aniline, *p*-chloroaniline, *m*-chloroaniline and 3,4-dichloroaniline were derivatized individually by method (b). Aniline produced many peaks and no further work was done with it. Table II shows the results of the derivatization of three substituted anilines. Varying the conditions of the reaction had little effect upon the reaction products of *p*-chloroaniline. Table III lists the heights of each of the peaks resulting from each of the three procedures tried. If the extract from the reaction of *p*-chloroaniline and pentafluorobenzoyl chloride was allowed to stand for several hours, the second peak decreased in height while the first peak increased correspondingly, perhaps indicating conversion of compound 2 to compound 1. Table IV illustrates this phenomenon.

**Table II. Gas chromatography of pentafluorobenzamide derivatives, using a 3% OV-1 column at 180°C, 60 ml/min carrier flow and an attenuation of 6 x 10**

| Parent aniline | pg inj | ret time (mm) | response (mm) |
|---|---|---|---|
| p-chloroaniline | 100 | 39 | 177 |
| | | 56 | 106 |
| m-chloroaniline | 100 | 39 | 110 |
| | | 49 | 78 |
| 3,4-dichloroaniline | 100 | 83 | 50 |

**Table III. Comparison of reaction products of *p*-chloroaniline under three different methods of derivatization**

| | Peak 1 | | Peak 2 | |
|---|---|---|---|---|
| Method | Ret (mm) | h (mm) | Ret (mm) | h (mm) |
| a) | 39 | 120 | 56 | 135 |
| b) | 39 | 159 | 56 | 139 |
| c) | 39 | 178 | 56 | 123 |

**Table IV. Effects of time on the pentafluorobenzamide derivatives of *p*-chloroaniline**

| Time | h, Peak 1 mm | h, Peak 2 mm |
|---|---|---|
| 0 | 159 | 139 |
| 3 hr. | 227 | 78 |
| 6 hr. | 218 | 84 |

The *p*-chloroaniline derivatives were subjected to silica gel chromatography using 1 g silica gel partially deactivated with 3% water. The extract was transferred to the column and eluted with 10 ml of each of the following solvents in order: 10% benzene in hexane, 30% benzene in hexane, 50% benzene in hexane, 80% benzene in hexane, benzene. Peak 1 was recovered principally in the benzene fraction (Fraction 5) while 77.5% of Peak 2 eluted with 30% benzene in hexane (Fraction 2) and 22.5% eluted with 50% benzene in hexane (Fraction 3). Recovery of the two peaks was complete.

It was postulated that the two peaks represented substitution of one nitrogen proton (Peak 1) and both nitrogen protons (Peak 2) but no further work was done nor is planned to provide additional characterization of these compounds.

Because of the obvious complications encountered with this reagent, it was not suitable for consideration as a means of aniline analysis and was pursued no further.

IV: *Substituted amines from pentafluorobenzyl bromide (PFB).* Prepare the PFB reagent by dissolving 42 µl of PFB in 1 ml of ethanol. Dissolve the aniline in acetone and pipette 1.0 ml into a 16 × 125 mm culture tube having a Teflon-lined screw cap. Add 0.055 ml of the reagent, cap tightly and heat for 2 hours in a 100°C heating block. Cool, add 5 ml of water, and extract successively with 5 ml, followed by three 2 ml portions of hexane. Dilute combined extracts to give a final concentration of about 25 ng/ml. Inject a volume containing 100-200 pg for GLC quantitation.

*Discussion:* The method described above was essentially the same as the method of Kawahara (9) for the derivation of acids and phenols. In that method, a little solid $K_2CO_3$ was used as a catalyst. If a mixture of anilines and phenols were derivatized in the presence of $K_2CO_3$, only phenol derivatives were produced. The same mixture in the absence of $K_2CO_3$ produced only aniline derivatives.

Recoveries of a mixture of aniline derivatives through silica gel (1 g silica gel, 3% water) were low, ranging from 58 to 77%. Complete recoveries were made from a column consisting of 2 g of alumina, basic, Woelm, partially deactivated with 5% (w/w) water. The column was eluted with 10 ml of hexane, which was discarded. The derivatives eluted with 10 ml of 50% benzene in hexane.

The gas chromatography of a solution of mixed aniline derivatives was very satisfactory. Good resolution of all compounds was accomplished using a 4% SE-30/6% OV-210 column at 190°C. Figure 4 shows a chromatogram of pentafluorobenzyl derivatives of substituted anilines.

V. *Substituted amines from nitro- and/or trifluoromethyl substituted phenyl halides.* Prepare derivatizing reagent solutions by dissolving 0.2 g of 1-fluoro-2,4-dinitrobenzene (FDNB), 4-chloro-α,α,α-trifluoro-3,5-dinitrotoluene (CDNT)

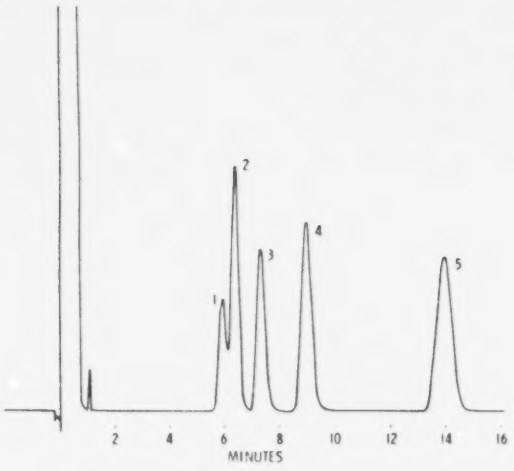



Figure 4. Chromatogram of pentafluorobenzyl derivatives of substituted anilines using a 6 ft 4% SE-30/ 6% OV-210 column at 190°C, 60 ml/min carrier flow and an attenuation of 16 x 10; 0.5 ng of 2) *p*-chloroaniline, 0.2 ng each of 1) *m*-chloroaniline, 3) 3-chloro-4-methylaniline, 4) *p*-bromoaniline, and 5) 3,4-dichloroaniline.

or $\alpha,\alpha,\alpha,4$-tetrafluoro-3-nitrotoluene (FMNT) in 10 ml of acetone. Pipette 1.0 ml of aniline standard in acetone into a 13 ml graduated centrifuge tube. Add 1 ml of derivatizing reagent, and 2 ml of 0.1 M $Na_2B_4O_7$ buffer. Mix and allow to stand for 2 hours at room temperature. Add 15 ml of water and extract with three 2 ml portions of methylene chloride. Dilute combined extracts to 10.0 ml, and add a little anhydrous $Na_2SO_4$. Evaporate a 1.0 ml aliquot just to dryness and dissolve the residue with hexane, diluting to an appropriate volume.

*Discussion:* Reaction occurred readily with FDNB and CDNT, but no significant reaction with FMNT was observed. The reaction took two hours to go to completion at room temperature. Heating at 55°C resulted in diminished yields. Dichloroaniline gave very poor yields with both CDNT and FDNB, apparently reacting slowly. At the end of four hours, the reaction of dichloroaniline with CDNT was still proceeding. Little improvement was noticed using buffers other than borate. Table V gives peak heights for the DNT derivative of dichloroaniline formed using different buffers.

The DNT derivatives were recovered through silica gel (1 g, 3% water) with 10 ml of 50% benzene in hexane. Recovery was complete for all compounds except dichloroaniline, which gave about 70% recovery. The dinitrophenyl derivatives were recovered with 10 ml of benzene.

**Table V. Effects of different buffers on the formation of the DNT derivative of dichloroaniline**

| Buffer | Molarity | pH | Pk ht (mm) |
|---|---|---|---|
| $NaH_2PO_4$ | 0.10 | 5.1 | 52 |
| $Na_2HPO_4$ | 0.10 | 9.0 | 90 |
| $Na_2HPO_4$ | 1.0 | 8.3 | 70 |
| $NaHCO_3$ | 0.10 | 8.4 | 77 |
| $Na_2B_4O_7$ | 0.10 | 9.2 | 79 |
| $Na_2CO_3$ | 0.10 | 11.6 | 91 |
| $Na_3PO_4$ | 0.10 | 12.0 | 111 |

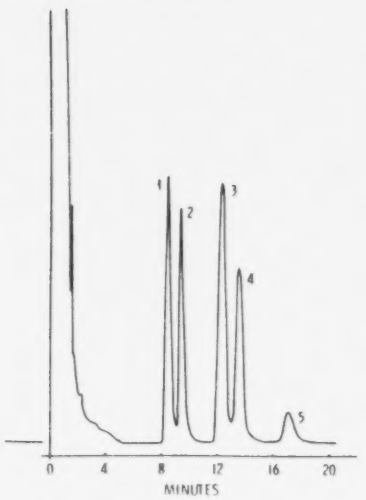



Figure 5. Chromatogram of 2,6-dinitro-4-trifluoromethylphenyl derivatives of substituted anilines using a 6 ft 3% OV-1 column at 200°C, 60 ml/min carrier flow and an attenuation of 16 x 10; 0.5 ng each of 1) *m*-chloroaniline, 2) *p*-chloroaniline, 3) 3-chloro-4-methylaniline and 4) *p*-bromoaniline and 1 ng of 5) 3,4-dichloroaniline.

The DNT derivatives were well resolved by GLC. Figure 5 shows a chromatogram of DNT derivatives of substituted anilines.

The 3% OV-1 column gave the best chromatography of dinitrophenyl derivatives of anilines, although 3-chloro-4-methylaniline and *p*-bromoaniline were not resolved. Other columns tried gave very broad and tailing peaks. Figure 6 shows a chromatogram of dinitrophenyl derivatives of substituted anilines.

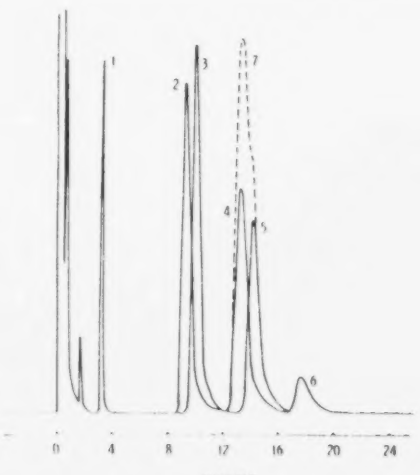
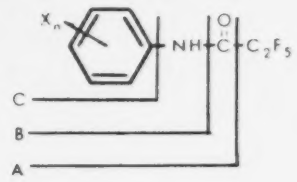



Figure 6. Chromatogram of 2,4-dinitrophenyl derivatives of substituted anilines using a 6 ft 3% OV-1 column at 200°C, 50 ml/min carrier flow and an attenuation of 16 x 10; 1 ng of 1) aniline, 2 ng each of 2) *m* chloroaniline, 3) *p* chloroaniline, 4) 3-chloro-4-methylaniline, 5) *p*-bromoaniline and 6) 3,4-dichloroaniline.

**Table VI. Significant Fragments from the Mass Spectra of Aniline Pentafluoropropionate Derivatives.**

**Figures in parentheses are the number of halogens in the fragment as indicated by halogen isotope cluster pattern and the relative height of the peak.**




| Parent Aniline | Molecular Ion $M^+$ | Fragments $M^+ - X$ | A | B | C | $C_2F_5$ | $CF_3$ |
|---|---|---|---|---|---|---|---|
| Aniline | 239 (100%) | | | 92 (75.3%) | 77 (95.2%) | 119 (25.5%) | 69 (30.2%) |
| m-chloro-aniline | 273 (Cl, 92.5%) | | 154 (Cl, 100%) | 126 (Cl, 69.1%) | 111 (Cl, 55.9%) | 119 (24.8%) | 69 (37.2%) |
| p-chloro-aniline | 273 (Cl, 98.6%) | | 154 (Cl, 67.1%) | 126 (Cl, 100%) | 111 (Cl, 46.4%) | 119 (18.8%) | 69 (48.2%) |
| p-bromo-aniline | 317 (Br, 75.7%) | | 198 (Br, 57.9%) | 170 (Br, 90.6%) | 155 (Br, 26.2%) | 119 (32.7%) | 69 (36.4%) |
| 3,4-dichloro-aniline | 307 (2Cl, 100%) | | 188 (2Cl, 69.4%) | 160 (2Cl, 83.7%) | 145 (2Cl, 21.9%) | 119 (20.9%) | 69 (29.9%) |
| 3,-chloro-4-methyl aniline | 287 (Cl, 100%) | 252 (12.3%) | 168 (Cl, 54.5%) | 140 (Cl, 62.8%) | 125 (Cl, 15.9%) | 119 (16.8%) | 69 (18.7%) |

*Selection of derivatization technique:* All derivatization reactions were run at 500 and 50 ng levels of each aniline. The derivative extracts were subjected to adsorption column chromatography for cleanup and elimination of as much interference as possible. In all cases, GC chromatograms of the higher level were acceptable. However, in most cases at the lower level, broad, tailing "solvent" peaks obscured the aniline derivative peaks. No way could be found to circumvent this difficulty. The only derivatization reagents which gave clean chromatograms at the low level were PFPA and HFBA, PFPA being the better of the two.

Although derivatives from these reagents did not have ideal GLC characteristics, it was decided to use PFPA as the derivatizing reagent in the hope that better columns could be found.

*Mass Spectroscopy:* Mass spectral data are consistent with the assigned structure of the PFP derivatives of anilines. Table VI shows the principle fragments found for each of the six compounds. Besides the molecular ion ($M^+$), these include fragments representing loss of a halogen ($M^+ - X$), cleavage on either side of the carbonyl (A and B) and cleavage between the nitrogen and aromatic ring (C). In addition, $C_2F_5^+$ and $CF_3^+$ fragments were conspicuous. The isotope cluster pattern for chloro- and bromo-substituted compounds were in agreement with the assigned structures.

*Recovery of Spikes:* Water and urine samples were spiked in triplicate at levels of 1.0 ppm and 0.10 ppm with mixtures of anilines. Mixtures were made up so that pairs of compounds that were not resolved by GLC were separated into different mixtures.

*Method:* Pipette 2.0 ml of sample into a 45 ml graduated conical centrifuge tube and add 0.1 ml of spiking mixture. Add 1 ml of 10% NaOH, 4 ml of benzene, and about 1 g of NaCl. Mix on a Vortex mixer for one minute, centrifuge five minutes at about 2,000 rpm to break the emulsion and separate the layers. Transfer the benzene extract to a 15 ml graduated centrifuge tube, reextract the aqueous layer twice with 2 ml portions of benzene and transfer extracts to the centrifuge tube. Evaporate under a stream of dry nitrogen to 0.2 ml. Add 0.2 ml of EtOAc and 0.1 ml of PFPA, mix, and allow to stand for one hour at room temperature. At the end of this time, add 2 ml of water and 5 ml of benzene. Mix on a Vortex mixer for one minute, centrifuge to separate layers, remove the aqueous layer with a disposable Pasteur pipette and discard. Wash the benzene layer twice with 2 ml portions of water, discarding the aqueous washes. Add a little anhydrous $Na_2SO_4$ to the benzene extract to remove traces of water. Evaporate carefully to about 1 ml and dilute to 5 ml with hexane to give a solution of derivatives in 20% benzene in hexane.

Prepare a silica gel column containing 1 g of silica gel (3% water), topped by 1 g of $Na_2SO_4$. Pre-wash with 10 ml of hexane, add the extract in benzene-hexane to the column. Rinse the tube with 5 ml of 20% benzene in hexane and add to the column. Discard eluates to this point. Place a 15 ml centrifuge tube under the column and elute with 15 ml of 60% benzene in hexane. Make proper dilutions or concentrations to give a final solution with a concentration of about 0.2 µg/10 ml.

Recoveries of anilines from water and urine spiked in triplicate at 1.0 ppm and 0.1 ppm were between 85 and 90%. Gas chromatograms were clean and easily quantitated. Further work is necessary to obtain good recoveries and clean chromatograms at 0.01 ppm.

Although one would like to have a little more sensitivity in this method, it still is a valuable tool in monitoring environmental and occupational exposure to those classes of pesticides which produce aniline urinary metabolites. The method is rapid and is readily applicable to a program involving analysis of large numbers of samples, such as one would encounter in a monitoring or clinical laboratory. The

sensitivity is sufficient for all but the lowest levels of exposure. Qualitative confirmation of identity of aniline metabolites is available by preparing any of the various other derivatives which, while not suitable for quantitative analysis, are ideal for confirmation. In short, this method should prove a valuable addition to the methodology available for monitoring human exposure to degradable pesticides.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